Distinct NMDA Receptor Subpopulations Contribute to Long-Term Potentiation and Long-Term Depression Induction

Sabina Hrabetova,[1] Peter Serrano,[1] Nancy Blace,[1] Heong W. Tse,[2] Donald A. Skifter,[3] David E. Jane,[2] Daniel T. Monaghan,[3] and Todd Charlton Sacktor[1]

[1]*Laboratory of Molecular Neuroscience, Departments of Physiology, Pharmacology, and Neurology, State University of New York, Downstate Medical Center, Brooklyn, New York 11203,* [2]*Department of Pharmacology, University of Bristol, School of Medical Sciences, Bristol BS81TD, United Kingdom, and* [3]*Department of Pharmacology, University of Nebraska Medical Center, Omaha, Nebraska 68198*

Long-term potentiation (LTP) and long-term depression (LTD) are persistent modifications of synaptic strength that have been implicated in learning, memory, and neuronal development. Despite their opposing effects, both forms of plasticity can be triggered by the activation of NMDA receptors. One mechanism proposed for this bidirectional response is that the specific patterns of afferent stimulation producing LTP and LTD activate to different degrees a uniform receptor population. A second possibility is that these patterns activate separate receptor subpopulations composed of different NMDA receptor (NR) subunits. To test this hypothesis we examined the inhibition of LTP and LTD by a series of competitive NMDA receptor antagonists that varied in their affinities for NR2A/B and NR2C/D subunits. The potency for the inhibition of LTP compared with inhibition of LTD varied widely among the agents. Antagonists with higher affinity for NR2A/B subunits relative to NRC/D subunits showed more potent inhibition of LTP than of LTD. D-3-(2-carboxypiperazine-4-yl)-1-propenyl-1-phosphonic acid, which binds to NR2A/B with very high affinity relative to NR2C/D, showed an ~1000-fold higher potency for LTP than for LTD. These results show that distinct subpopulations of NMDA receptors characterized by different NR2 subunits contribute to the induction mechanisms of potentiation and depression.

Key words: NMDA; NR2 subunit; long-term potentiation; long-term depression; D-3-(2-carboxypiperazine-4-yl)-1-propenyl-1-phosphonic acid; D-2-amino-5-phosphonovaleric acid; (±)-cis-*1-(phenanthren-2-yl-carbonyl)piperazine-2,3-dicarboxylic acid*

In the hippocampal CA1 region and the cerebral cortex, both long-term potentiation (LTP) and long-term depression (LTD) can depend on the activation of NMDA receptors, because both can be blocked by the NMDA receptor antagonist D/L-2-amino-5-phosphonovaleric acid (D/L-AP5) (Collingridge et al., 1983; Harris et al., 1984; Dudek and Bear, 1992; Mulkey and Malenka, 1992; Kirkwood et al., 1993; Christie et al., 1996; Cummings et al., 1996). High-frequency stimulation causes strong activation of the ligand- and voltage-dependent NMDA receptors and a large influx of Ca^{2+} into postsynaptic neurons to trigger potentiation. Low-frequency stimulation results in moderate activation of NMDA receptors and a moderate influx of Ca^{2+}, leading to depression. An additional mechanism participating in this bidirectional response, however, may be that high- and low-frequency stimulation activate distinct subpopulations of NMDA receptors (Hrabetova and Sacktor, 1997).

NMDA receptors consist of NMDA receptor 1 (NR1) subunits and members of a family of glutamate-binding NR2 subunits (NR2A–D) (Ikeda et al., 1992; Monyer et al., 1992; Ishii et al., 1993). Recombinant NMDA receptors that contain NR1 subunits and subunits NR2A or B require a strong depolarization to overcome Mg^{2+} blockade and have high conductances, and those with NR2C or D need only modest depolarization to overcome Mg^{2+} blockade and show low conductances (Monyer et al., 1992, 1994). Native NMDA receptors containing NR2D are estimated to form ~10% of the NMDA receptor population in the cortex of adult rats (Dunah et al., 1998), and levels of expression of the subunits are higher in juvenile animals (Dunah et al., 1996; Wenzel et al., 1996), when LTD can most efficiently be produced (Dudek and Bear, 1993). CA1 pyramidal cells express mRNA for NR2A, 2B, and 2D in adult humans (Scherzer et al., 1998) and in juvenile rats (Kirson et al., 1999). Currents attributable to NMDA receptors containing NR2D subunits have been observed in juvenile CA1 pyramidal cells (Kirson et al., 1999).

NMDA receptor subpopulations containing these different subunits can be distinguished by competitive NMDA receptor antagonists with different affinities to the glutamate-binding site of the various NR2s (Monaghan et al., 1998). Comparisons of LTP and LTD using these antagonists, however, must take into account the receptor occupancies required for LTP and LTD induction, which are not known and may not be the same. LTP, for example, may require the agonist occupation of a high percentage of receptors for a large increase in postsynaptic Ca^{2+},

Received Feb. 7, 2000; revised March 29, 2000; accepted April 11, 2000.

This research was supported by Grants RO1 57068 and R29/RO1 53576 from the National Institute of Mental Health (T.C.S.) and Grant 17-94-C-4050 from the US Army Medical Research Division (D.T.M.). We thank Dr. Jan Hrabe for statistical analysis and Dr. Feng Bihua for assistance with autoradiograms. We thank Drs. Peter Bergold, Stanley Friedman, Robert Muller, Wayne Sossin, Keith Williams, and Robert K. S. Wong for discussion and comments.

Correspondence should be addressed to Dr. Todd C. Sacktor, Laboratory of Molecular Neuroscience, Box 29, Departments of Physiology, Pharmacology, and Neurology, State University of New York, Downstate Medical Center, 450 Clarkson Avenue, Brooklyn, NY 11203. E-mail: TSACKTOR@netmail.hscbkyn.edu.

Dr. Hrabetova's present address: Department of Physiology and Neuroscience, New York University Medical Center, 550 First Avenue, New York, NY 10016.

This article is published in *The Journal of Neuroscience*, Rapid Communications Section, which publishes brief, peer-reviewed papers online, not in print. Rapid Communications are posted online approximately one month earlier than they would appear if printed. They are listed in the Table of Contents of the next open issue of JNeurosci. Cite this article as: JNeurosci, 2000, 20:RC81 (1–6). The publication date is the date of posting online at www.jneurosci.org.

http://www.jneurosci.org/cgi/content/full/4274

Table 1. K_i values for recombinant NMDA receptors containing NR2 subunits A–D and IC_{50} values for LTP and LTD of the NMDA receptor antagonists PPDA, D-AP5, and D-CPPene

	PPDA	D-AP5	D-CPPene
NR2A/NR1 K_i (μM)	0.68 ± 0.17	0.28 ± 0.02	0.11 ± 0.03
NR2B/NR1 K_i (μM)	0.35 ± 0.02	0.46 ± 0.14	0.14 ± 0.04
NR2C/NR1 K_i (μM)	0.070 ± 0.015	1.64 ± 0.14	1.46 ± 0.08
NR2D/NR1 K_i (μM)	0.108 ± 0.032	3.71 ± 0.67	1.84 ± 0.74
(2C + 2D) K_i/(2A + 2B) K_i	0.17	7.2	13.2
(2C + 2D) K_i/(2A + 2B) $K_i^{nl\ PPDA}$	1	42	78
LTP IC_{50} (μM)	0.29	0.014	0.022
LTD IC_{50} (μM)	0.63	0.45	25.3
LTD IC_{50}/LTP IC_{50}	2	32	1150
LTD IC_{50}/LTP $IC_{50}^{nl\ PPDA}$	1	16	575

The rank order of decreasing affinity for recombinant 2C/D receptors, relative to 2A/B receptors, matches the decreasing potency for LTD, relative to LTP. K_i values were obtained from NR2 subunits recombinantly expressed with NR1a subunits to form receptors in *Xenopus* oocytes. The K_i values for D-AP5 and D-CPPene are from Buller and Monaghan (1997) (mean ± SEM; $n = 4–6$); the K_i values for PPDA were from B. Feng, H. W. Tse, D. A. Skifter, D. E. Jane, and D. T. Monaghan (unpublished data) ($n = 3$). Differences among the agents were expressed by normalization to PPDA [(2C + 2D) K_i/(2A + 2B) $K_i^{nl\ PPDA}$ and LTD IC_{50}/LTP $IC_{50}^{nl\ PPDA}$], obtained by dividing the ratios for each drug by the ratio for PPDA.

whereas LTD might require occupation of only a few receptors for a modest rise in Ca^{2+} (Lisman, 1989; Artola and Singer, 1993; Hansel et al., 1997; Yang et al., 1999). If this were the case, more receptors would need to be blocked to prevent depression than potentiation, and all NMDA receptor antagonists would show a higher IC_{50} for LTD than for LTP. When comparing antagonists that vary in their NR2 subunit selectivity, the ratio of LTD IC_{50} to LTP IC_{50} would be similar for all agents if a single receptor subtype mediated both forms of plasticity but would differ if specific subunits selectively contributed to LTP or LTD.

In this study, we find large differences in the potencies of the antagonists for inhibition of LTP and LTD, indicating that NMDA receptors with distinct subunit compositions contribute to the induction mechanisms of LTP and LTD.

Portions of this paper were published previously in abstract form (Hrabetova et al., 1998).

MATERIALS AND METHODS

Immunoblots. Immunoblots of membrane fractions from rat CA1 regions at different developmental ages were performed as previously described (Hrabetova and Sacktor, 1996). Rabbit antiserum to NR2A/B (used at 1:1000) was a generous gift from Dr. R. J. Wenthold (National Institutes of Health, Bethesda, MD); rabbit antiserum to NR2D (1:50) was a generous gift from Dr. B. B. Wolfe (Georgetown University School of Medicine, Washington, DC); rabbit antisera to NR2C (1:200) was from Chemicon (Temecula, CA). Total protein per lane was: NR2A/B, 10 μg/lane; 2C, 40 μg/lane for CA1, 50 μg/lane for thalamus, and 30 μg/lane for cerebellum; and 2D, 40 μg/lane.

In situ *hybridization.* NR2D mRNA was visualized as previously described (Buller et al., 1994). Freshly frozen Sprague Dawley rat (11-d-old) brains were sectioned (12 μm), thaw-mounted, and fixed for 5 min in 4% paraformaldehyde at 4°C. After ethanol dehydration, a 45 mer ^{35}S-labeled antisense NR2D (5′-CTCCGAATCCTCGGAGTCCGAAG-GCGAAGGCTCGAGGTCCAGGTA-3′), complementary to sequences encoding amino acids 1067–1081 of the ϵ4 subunit (Ikeda et al., 1992), was dissolved in hybridization buffer (2000 cpm/μl, ~0.2 nM; New England Nuclear, Boston, MA) containing 0.2 M dithiothreitol and applied to sections for overnight incubations under Parafilm-sealed coverslips at 42°C and then washed for 20 min at a final stringency of 1× SSC (0.015 M sodium citrate and 0.15 M NaCl) at 60°C. Sections were air-dried and exposed to film (βMax; Amersham, Arlington Heights, IL). Probe specificity was confirmed by incubation of the radiolabeled oligonucleotides with 100 nM unlabeled probe (data not shown).

In vitro *transcription and translation in* Xenopus *oocytes.* RNA translation and transcription in *Xenopus* oocytes and electrophysiological recordings were performed as previously described (Monaghan and Larson, 1997). cDNA encoding the NR1a subunit was a generous gift from Dr. S. Nakanishi (Kyoto University, Faculty of Medicine, Kyoto, Japan); NR2A, NR2C, and NR2D subunits were generous gifts from Dr. P. Seeburg (University of Heidelberg, Heidelberg, Germany); NR2B was generously provided by Dr. D. Pritchett and Dr. D. Lynch (University of Pennsylvania, Philadelphia, PA). Plasmids were linearized with *Not*I (NR1a), *Eco*RI (NR2A, NR2C, and NR2D), or *Sal*I (NR2B) and transcribed *in vitro* using the mMessage mMachine RNA polymerase transcription kit (Ambion, Austin, TX). NR1a was mixed 1:3 with NR2A, NR2B, NR2C, or NR2D RNA, and 2–50 ng of this mixture was injected into oocytes. Agonist-evoked responses were measured using a standard two-microelectrode voltage clamp (model OC-725B Oocyte Clamp; Warner Instruments, Hamden, CT) at a holding potential of −60 mV. Glutamate (10 μM) and glycine (10 μM) were applied until stable plateau responses were obtained; (±)-cis-1-(phenanthren-2yl-carbonyl)-piperazine-2,3-dicarboxylic acid (PPDA) (0.1, 0.3, 1, 3, 10, or 30 μM) was then applied until steady-state blockade was obtained, followed by antagonist washout and full agonist responses. Current responses were captured and analyzed with AxoData (Axon Instruments, Foster City, CA) and GraphPad Prism (ISI Software, San Diego, CA) software. K_i values were corrected for agonist affinity according to the Cheng–Prusoff equation. Data in Table 1 are expressed as mean K_i ± SEM.

PPDA was found to have little effect on native non-NMDA glutamate receptors. Autoradiography, performed as described by Monaghan (1993), showed that at 10 μM, PPDA inhibited 44.6 ± 1.3% of 100 nM [^{3}H]CNQX binding to native AMPA receptors and 9.3 ± 6.7% of 25 nM [^{3}H]kainate ($n = 3$).

Electrophysiology. Transverse hippocampal slices (450 μm) were prepared as previously described (Hrabetova and Sacktor, 1996). Briefly, Sprague Dawley rats (16–21 d) were anesthetized with halothane and decapitated. The hippocampi were dissected, bathed in cold saline, and sliced with a McIlwain tissue slicer. Slices were placed in an interface recording chamber and kept for 30 min in a saline solution containing 125 mM NaCl, 2.5 mM KCl, 1.25 mM NaH_2PO_4, 26 mM $NaHCO_3$, 11 mM glucose, 10 mM $MgCl_2$, and 0.5 mM $CaCl_2$, pH 7.4, equilibrated with 95% O_2 and 5% CO_2 at 32°C. The slices were then perfused with the saline solution containing 1.2 $MgCl_2$, and 1.7 mM $CaCl_2$. Test stimuli (100 μsec) were delivered every 15 sec through bipolar tungsten electrodes placed across the Schaffer collateral–commissural fibers. Field EPSPs were recorded using glass microelectrodes filled with the saline solution (resistance, 5–10 MΩ) and placed in CA1 stratum radiatum. The current intensity of test stimuli (25–50 μA) was set to produce half-maximal EPSPs (2–3 mV). The baseline was recorded for at least 10 min to ensure the stability of the response. LTP was induced by a 100 Hz, 1 sec train at a current set to produce 75% of the maximal EPSP response. LTD was induced by 3 Hz stimulation for 5 min at the current intensity of the test stimulus. Data were collected and analyzed using Superscope (GW Instrument, Somerville, MA). The slope of the field EPSP was measured beginning at 10% and ending at 50% of the initial phase of the EPSP response. PPDA was synthesized by a method that will be published elsewhere; D-AP5 was obtained from Tocris Cookson (St. Louis, MO); D-3-(2-carboxypiperazine-4-yl)-1-propenyl-1-phosphonic acid (D-CPPene) was a generous gift from Novartis (Berne, Switzerland). The compounds were added to the bath at least 40 min before the induction of LTP or LTD. Dose–response curves for the effect of NMDA receptor antagonists on the efficacy of LTP and LTD were fitted (Mathematica; Wolfram Research, Inc., Champaign, IL) according to the equation $E = E_{max} - E_{max}/[1 + (IC_{50}/A)^n]$, where E is the efficacy of LTP or LTD in the presence of antagonist, E_{max} is the control efficacy of LTP or LTD in the absence of antagonist, IC_{50} is the concentration of antagonist half inhib-

iting the maximal response, A is the antagonist concentration, and n is the Hill coefficient.

RESULTS

To establish the presence of the various NR2 subunits in the CA1 region of the rat hippocampus, we first examined the developmental expression of the subunits by immunoblot. The expression of NR2A/B increased during development from juvenile to adult ages (Fig. 1*A*). In contrast, NR2D was maximal at 2 weeks and declined slightly in adulthood (Fig. 1*A*), a result consistent with other reports of NR2D protein levels in whole rat brain (Wenzel et al., 1996) and telencephalon (Dunah et al., 1996). Low levels of 2C protein were also found in the CA1 of juvenile animals (Fig. 1*A*). The expression of the NR2D mRNA was localized to the CA1 pyramidal cell layer by *in situ* hybridization (Fig. 1*B*).

A series of NMDA receptor antagonists that differ in their binding affinities for NR2A/B and NR2C/D subunits, PPDA, D-AP-5, and D-CPPene, was then used to examine LTP and LTD induction (Table 1). LTP was induced by a single train of 100 Hz lasting 1 sec applied to Schaffer collateral–commissural fibers in the CA1 region of the hippocampus, and LTD was produced by 3 Hz stimulation for 5 min (Fig. 2*A*). The effect on synaptic transmission was measured as a change in the initial slope of EPSPs recorded extracellularly in stratum radiatum. Without antagonists, 30 min after the 100 Hz stimulation, the synaptic responses were 168.9 ± 7.5% of baseline (set at 100% ± SEM; Fig. 2*A*). Thirty minutes after the 3 Hz stimulation, the synaptic efficacy decreased to 65.5 ± 3.9% of baseline (Fig. 2*A*). For each dose of antagonist examined, we demonstrated normal LTP or LTD in adjacent slices from the same hippocampus in physiological saline, as controls. Inhibition was determined as the difference in potentiation or depression at 30 min in the presence and absence of the drug (n = 4–6 slices for each drug concentration and for paired controls).

PPDA is an NMDA receptor antagonist with 3- to 10-fold higher affinity for recombinant NR2C/D subunits than for NR2A/B subunits (Table 1). Neither LTP nor LTD was blocked with 0.1 μM

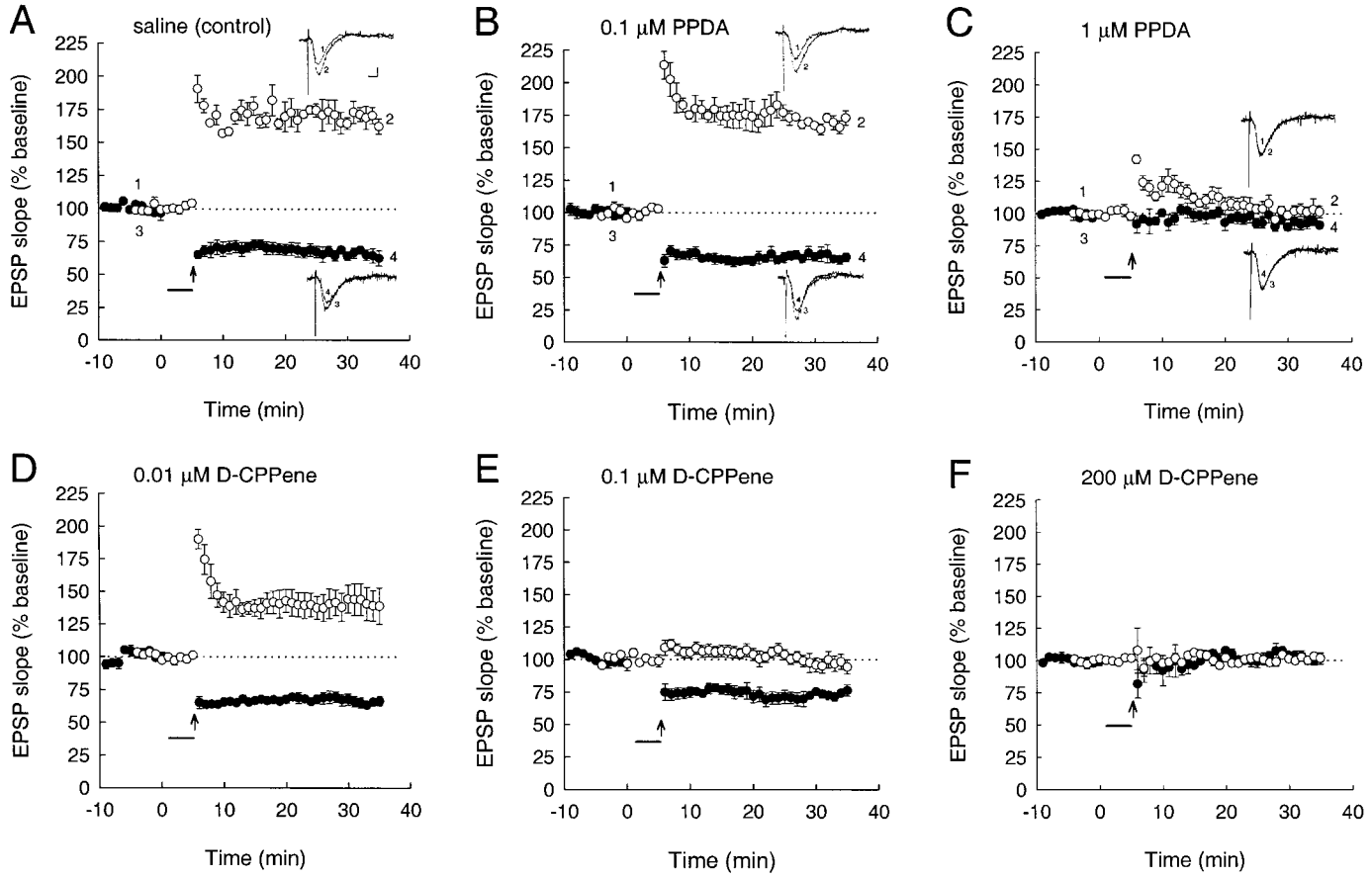

Figure 2. Dose responses for LTP and LTD induction of the NMDA receptor antagonists PPDA, D-AP5, and D-CPPene. Representative field EPSPs correspond to *numbered points* in the time courses (calibration: 1 mV vertically, 5 msec horizontally). *A*, LTP (*open circles*) is induced by 100 Hz stimulation lasting 1 sec (*arrow*). LTD (*closed circles*) is induced by 3 Hz stimulation applied for 5 min (*short horizontal bar*). *B*, 0.1 μM PPDA does not alter LTP or LTD. *C*, 1 μM PPDA blocks both LTP and LTD. *D*, 0.01 μM D-CPPene does not block the induction of LTP or LTD. *E*, 0.1 μM D-CPPene blocks LTP but has no effect on LTD. *F*, 200 μM D-CPPene blocks both LTP and LTD. Field EPSPs are normalized to baseline set at 100% ± SEM; $n = 4–6$ for all experiments.

tivation of conventional NR2A/B-containing NMDA receptors, which require high-frequency stimulation to overcome their high degree of Mg^{2+} block, contribute to LTP, whereas NR2C/D-containing NMDA receptors, which require relatively low levels of depolarization to overcome their Mg^{2+} block (Monyer et al., 1992, 1994), contribute to LTD. Our results are consistent with earlier observations that the NMDA receptor antagonists (5*R*,10*S*)-(+)-5-methyl-10-11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine (MK801) and 3-((*R*,*S*)-2-carboxypiperazin-4-yl)propyl-1-phosphonic acid (CPP), both of which have a preference for NR2A/B over NR2C/D subunits (Beaton et al., 1992; Buller et al., 1994), effectively block LTP (Abraham and Mason, 1988; Mayford et al., 1995) but do not generally block LTD (Mayford et al., 1995; Hrabetova and Sacktor, 1997) (although see Heynen et al., 1996). Because the stoichiometry of subunits in native NMDA receptors is not yet known, different ratios of the NR2 subunits may determine the distinct receptor subpopulations contributing to potentiation and depression.

The differences in LTD/LTP IC_{50} values among the series of agents was large. For D-CPPene, the magnitude of the difference in LTD and LTP IC_{50} values was greater than that of the difference in affinities for the recombinantly expressed receptors (Table 1). This is likely because of a stronger ability of the agent to distinguish subunits in their native states compared with subunits that are expressed recombinantly. For example, the binding of D-CPPene to low-affinity sites in the cerebellum, attributable to native NR2C-containing receptors, has a K_i of 14.3 ± 3.6 μM (Buller et al., 1994), whereas its binding to high-affinity native sites, attributable to NR2A-containing receptors, has a K_i of 0.25 ± 0.06 μM. PPDA has a K_i of 0.39 μM to these native 2C receptors and 7.2 μM to the native 2A receptors (D. T. Monaghan, unpublished observations). This would result in a 1057-fold difference between D-CPPene and PPDA in the ability to distinguish these native receptors ($[K_i^{\text{D-CPPene}}2C/K_i^{\text{D-CPPene}}2A]/[K_i^{\text{PPDA}}2C/K_i^{\text{PPDA}}2A]$), comparable with the 575-fold difference in LTD/LTP IC_{50} values observed for the two agents. These differences between native and recombinant receptors, likely to arise from subunit composition and post-translational modifications (Grimwood et al., 1993), may also contribute to the differences in the absolute values between the K_i values for the recombinant receptors and the IC_{50} values of LTD and LTP.

Although D-CPPene showed orders of magnitude difference in LTD and LTP potencies, PPDA, the antagonist with the strongest preference for NRC/D subunits tested, still showed a twofold higher IC_{50} for LTD than for LTP. The absolute IC_{50} values, however, may be determined, in part, by differences in the occupancy of receptor subpopulations required for LTP and LTD induction, such that a higher percentage of NR2C/D-containing receptors must be blocked to prevent LTD, and a lower percentage of NR2A/B-containing receptors must be blocked to prevent LTP.

The contribution of NR2C/D-containing receptors to synaptic depression was observed with both low-frequency afferent stimulation and high-frequency stimulation in which LTP was prevented by the NR2A/B-selective antagonist D-CPPene (Fig. 4). A

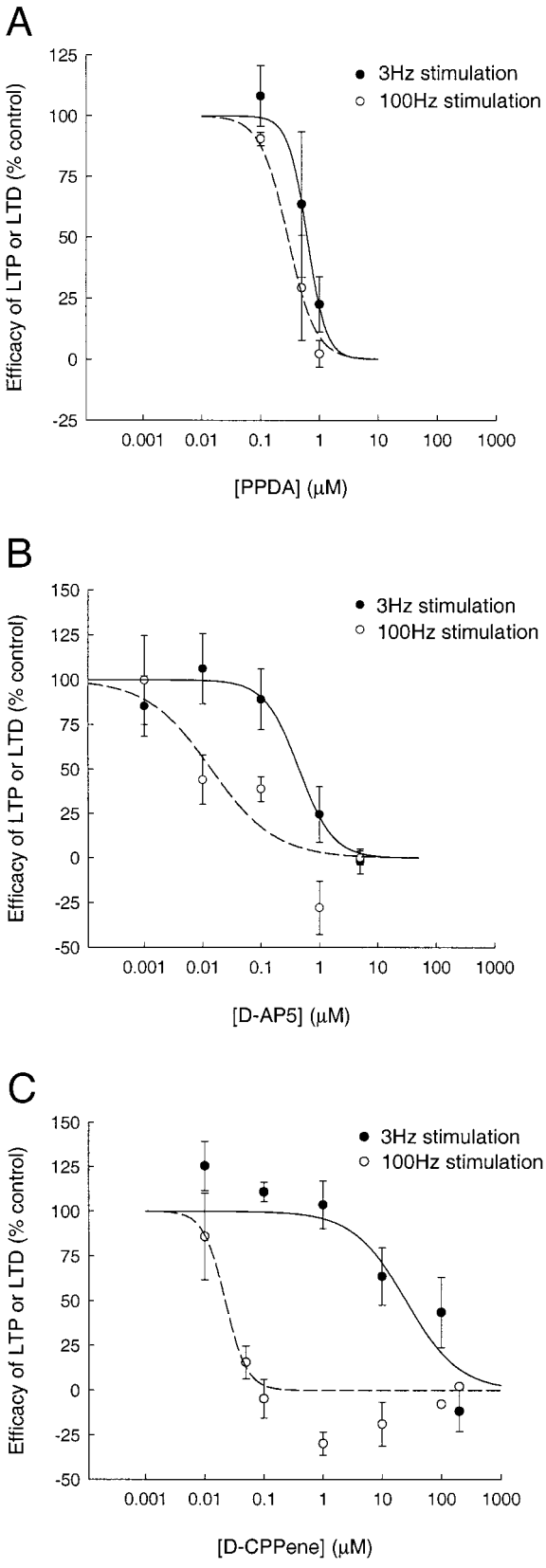

Figure 3. PPDA, D-AP5, and D-CPPene have different LTD/LTP IC_{50} ratios. *A*, PPDA shows a similar IC_{50} for 3 Hz LTD (*closed circles*) and 100 Hz LTP (*open circles*). *B*, D-AP5 has a moderately higher IC_{50} for LTD than for LTP. *C*, D-CPPene shows a ~1000-fold higher IC_{50} for LTD than for LTP.

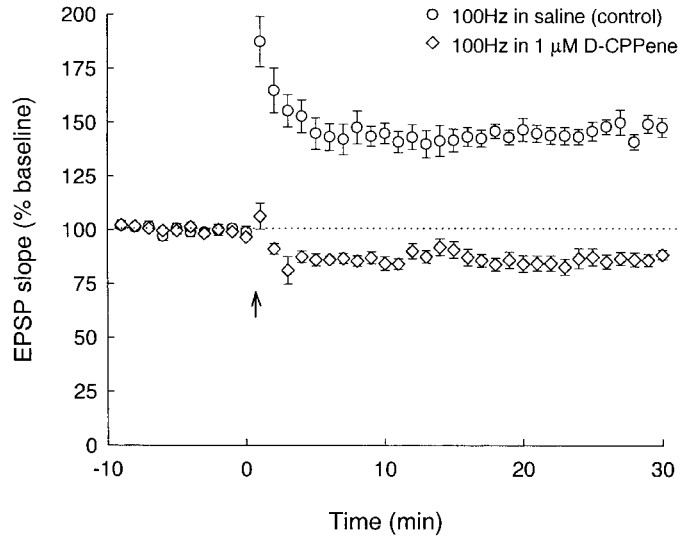

Figure 4. High-frequency, 100 Hz stimulation produces LTD in the presence of 1 μM D-CPPene (*open diamonds*; $n = 8$), a concentration that blocks 100 Hz LTP but not 3 Hz LTD. Control LTP in adjacent hippocampal slices was observed in drug-free saline (*open circles*).

nearly identical result has been reported for adult rats injected with moderate doses of CPP, in which prime-burst, high-frequency stimulation used to induce *in vivo* hippocampal LTP was found to produce *in vivo* LTD (Kentros et al., 1998). A similar result was also obtained in slices bathed in a moderate dose of D-AP5, in which tetanic stimulation that normally produces LTP was found to produce LTD (Cummings et al., 1996). These results are consistent with the interpretation that low-frequency stimulation initiates the molecular mechanisms only for depression, whereas high-frequency stimulation activates the mechanisms of both potentiation and depression, but the former masks or outweighs the latter.

Our results show that the molecular mechanisms of potentiation and depression diverge at the very beginning of postsynaptic signal transduction through distinct NMDA subpopulations. Differences in conductances and the duration of opening between two receptor populations may contribute to the regulation of postsynaptic Ca^{2+} influx in the induction of LTP and LTD (Lisman, 1989; Artola and Singer, 1993; Malenka, 1994; Hansel et al., 1997). The relatively low conductance for cations and the long duration of opening of the 2D channel (Monyer et al., 1994), for example, may permit the modest but prolonged Ca^{2+} influx that has been reported to be required to trigger the induction of LTD (Yang et al., 1999). Our results do not indicate the mechanism by which distinct NMDA subpopulations produce particular signal transduction pathways, leading ultimately to persistent strengthening or weakening of synaptic efficacy. Two hypotheses offer equally plausible mechanisms. Ca^{2+} signals produced by the different receptor populations may result in different biochemical pathways based only on their concentration and time course. Alternatively, these signals may have unique spatiotemporal properties and preferential access to particular downstream signaling molecules. NR2C and 2D, for example, possess a proline-rich intracellular C-terminal domain (Ikeda et al., 1992; Ishii et al., 1993), which, in the case of 2D, allows for the selective association with the Src homology 3 domain of c-ABL kinase (D. Monaghan, personal communication). By dividing the functions of the NMDA receptor into two distinct receptor subpopulations, LTP and LTD induction can be independently regulated, increasing the flexibility of the bidirectional regulation of synaptic strength.

REFERENCES

Abraham WC, Mason SE (1988) Effects of the NMDA receptor/channel antagonists CPP and MK801 on hippocampal field potentials and long-term potentiation in anesthetized rats. Brain Res 462:40–46.

Artola A, Singer W (1993) Long-term depression of excitatory synaptic transmission and its relationship to long-term potentiation. Trends Neurosci 16:480–487.

Beaton JA, Stemsrud K, Monaghan DT (1992) Identification of a novel *N*-methyl-D-aspartate receptor population in the rat medial thalamus. J Neurochem 59:754–757.

Buller AL, Monaghan DT (1997) Pharmacological heterogeneity of NMDA receptors: characterization of NR1a/NR2D heteromers expressed in *Xenopus* oocytes. Eur J Pharmacol 320:87–94.

Buller A, Larson H, Schneider B, Beaton J, Morrisett R, Monaghan D (1994) The molecular basis of NMDA receptor subtypes: native receptor diversity is predicted by subunit composition. J Neurosci 14:5471–5484.

Christie BR, Magee JC, Johnston D (1996) The role of dendritic action potentials and Ca^{2+} influx in the induction of homosynaptic long-term depression in hippocampal CA1 pyramidal neurons. Learn Mem 3:160–169.

Collingridge GL, Kehl SJ, McLennan H (1983) Excitatory amino acids in synaptic transmission in the Schaffer collateral-commissural pathway of the rat hippocampus. J Physiol (Lond) 334:34–46.

Cummings JA, Mulkey RM, Nicoll RA, Malenka RC (1996) Ca^{2+} signaling requirements for long-term depression in the hippocampus. Neuron 16:825–833.

Dudek SM, Bear MF (1992) Homosynaptic long-term depression in area CA1 of hippocampus and effects of *N*-methyl-D-aspartate receptor blockade. Proc Natl Acad Sci USA 89:4363–4367.

Dudek SM, Bear MF (1993) Bidirectional long-term modification of synaptic effectiveness in the adult and immature hippocampus. J Neurosci 13:2910–2918.

Dunah AW, Yasuda RP, Wang YH, Luo J, Davila-Garcia M, Gbadegesin M, Vicini S, Wolfe BB (1996) Regional and ontogenic expression of the NMDA receptor subunit NR2D protein in rat brain using a subunit-specific antibody. J Neurochem 67:2335–2345.

Dunah AW, Luo J, Wang YH, Yasuda RP, Wolfe BB (1998) Subunit composition of *N*-methyl-D-aspartate receptors in the central nervous system that contain the NR2D subunit. Mol Pharmacol 53:429–437.

Grimwood S, Wilde GJC, Foster AC (1993) Interactions between the glutamate and glycine recognition sites of the *N*-methyl-D-aspartate receptor from rat brain, as revealed from radioligand binding studies. J Neurochem 60:1729–1738.

Hansel C, Artola A, Singer W (1997) Relation between dendritic Ca^{2+} levels and the polarity of synaptic long-term modifications in rat visual cortex neurons. Eur J Neurosci 9:2309–2322.

Harris EW, Ganong AH, Cotman CW (1984) Long-term potentiation in the hippocampus involves activation of *N*-methyl-D-aspartate receptors. Brain Res 323:132–137.

Heynen AJ, Abraham WC, Bear MF (1996) Bidirectional modifications of CA1 synapses in the adult hippocampus in vivo. Nature 381:163–166.

Hrabetova S, Sacktor TC (1996) Bidirectional regulation of protein kinase Mζ in the maintenance of long-term potentiation and long-term depression. J Neurosci 16:5324–5333.

Hrabetova S, Sacktor TC (1997) Long-term potentiation and long-term depression are induced through pharmacologically distinct NMDA receptors. Neurosci Lett 226:107–110.

Hrabetova S, Monaghan D, Sacktor TC (1998) LTP and LTD are induced through two distinct NMDA-receptor subpopulations. Eur J Neurosci 10[Suppl 10]:26.

Ikeda K, Nagasawa M, Mori H, Araki K, Sakimura K, Watanabe M, Inoue Y, Mishina M (1992) Cloning and expression of the epsilon 4 subunit of the NMDA receptor channel. FEBS Lett 313:34–38.

Ishii T, Moriyoshi K, Sugihara H, Sakurada K, Kadotani H, Yokoi M, Akazawa C, Shigemoto R, Mizuno N, Masu M, Nakanishi S (1993) Molecular characterization of the family of the *N*-methyl-D-aspartate receptor subunits. J Biol Chem 268:2836–2843.

Kentros C, Hargreaves E, Hawkins RD, Kandel ER, Shapiro M, Muller RV (1998) Abolition of long-term stability of new hippocampal place cell maps by NMDA receptor blockade. Science 280:2121–2126.

Kirkwood A, Dudek SM, Gold JT, Aizenman CD, Bear MF (1993) Common forms of synaptic plasticity in the hippocampus and neocortex in vitro. Science 260:1518–1521.

Kirson ED, Schirra C, Konnerth A, Yaari Y (1999) Early postnatal switch in magnesium sensitivity of NMDA receptors in rat CA1 pyramidal cells. J Physiol (Lond) 521:99–111.

Lisman J (1989) A mechanism for the Hebb and the non-Hebb processes underlying learning and memory. Proc Natl Acad Sci USA 86:9574–9578.

Malenka RC (1994) Synaptic plasticity in the hippocampus: LTP and LTD. Cell 78:535–538.

Mayford M, Wang J, Kandel E, O'Dell T (1995) CaMKII regulates the frequency-response function of hippocampal synapses for the production of both LTD and LTP. Cell 81:891–904.

Monyer H, Sprengel R, Schoepfer R, Herb A, Higuchi M, Lomeli H, Burnashev N, Sakmann B, Seeburg P (1992) Heteromeric NMDA receptors: molecular and functional distinction of subtypes. Science 256:1217–1221.

Monyer H, Burnashev N, Laurie D, Sakmann B, Seeburg P (1994) Developmental and regional expression in the rat brain and functional properties of four NMDA receptors. Neuron 12:529–540.

Monaghan DT (1993) Autoradiographic analysis of excitatory amino acid receptors. In: Receptor autoradiography: principles and practice (Wharton J, Polak JM, eds), pp 171–193. New York: Oxford UP.

Monaghan DT, Larson H (1997) NR1 and NR2 subunit contributions to NMDA receptor channel blocker pharmacology. J Pharmacol Exp Ther 280:614–620.

Monaghan DT, Andalora VJ, Skifter D (1998) Molecular determinants of NMDA receptor pharmacological diversity. Prog Brain Res 116:158–177.

Mulkey RM, Malenka RC (1992) Mechanisms underlying induction of homosynaptic long-term depression in area CA1 of the hippocampus. Neuron 9:967–975.

Scherzer CR, Landwehrmeyer GB, Kerner JA, Counihan TJ, Kosinski CM, Standaert DG, Daggett LP, Velicelebi G, Penney JB, Young AB (1998) Expression of *N*-methyl-D-aspartate receptor subunit mRNAs in the human brain: hippocampus and cortex. J Comp Neurol 390:75–90.

Wenzel A, Villa M, Mohler H, Benke D (1996) Developmental and regional expression of NMDA receptor subtypes containing the NR2D subunit in rat brain. J Neurochem 66:1240–1248.

Yang SN, Tang YG, Zucker RS (1999) Selective induction of LTP and LTD by postsynaptic $[Ca^{2+}]$i elevation. J Neurophysiol 81:781–787.